Technical Abstract

Our long term **goal** is to develop an effective intraperitoneal adoptive immunotherapy strategy f ovarian carcinoma utilizing TIL-(tumor infiltrating lymphocytes) derived T cells expanded in lc concentrations of rIL-2. To achieve this goal it is important to determine whether ovarian T concentrate at metastatic tumor sites in vivo. Gene transfer technology offers a sensitive ar reliable method to track TIL to tumor sites over a prolonged period of time. The specific **objectiv** are to determine: (1) The feasibility of detecting $CD3^+CD8^+$ TIL-derived T cells that have be marked with the gene for Neomycin phosphotransferase encoded in a safety modified retrovir vector (G1Na) at tumor sites after IP injection, and (2) The fold of enrichment of $CD3^+CD8^+$ mark TIL at tumor sites.

Background and Rationale

Epithelial ovarian cancer primarily involves the peritoneal and serosal surfaces, a fact that h made this tumor a target for intraperitoneal (IP) biologic therapies including adoptive cellul therapy (1,2). Preclinical studies from our laboratory suggest that ovarian TIL may represent a active immune response of the host directed against the tumor (3,4). T-cell lines from ovarian T expanded in low dose rIL-2 exhibited cytotoxicity primarily against autologous ovarian tumor cel Our studies have shown that the TCR CD3 complex and CD8 antigens on rIL-2 expanded TIL, ar MHC Class I surface antigens on ovarian carcinoma cells are involved in this in vitro cytotox activity (3,4). Ovarian TIL-derived T-cell lines have also been shown to produce substanti quantities of cytokines including interferon-γ, TNF-α, and GMCSF following culture in lc concentrations of rIL-2 (20 IU) (5). Production of these cytokines by $CD8^+$ T-cell lines can b significantly enhanced by stimulation with autologous tumor cells. TIL may have therapeutic effe in vivo either by direct cell to cell contact or through the production of the cytokines describ above. We have completed a pilot clinical protocol in which 8 patients with advanced platinu refractory ovarian carcinoma received IP rIL-2 expanded TIL and low dose rIL-2. Four of the patients had clinical activity after receiving IP TIL plus low dose rIL-2. In a preliminary stuc utilizing ^{111}In-labeled rIL-2 expanded TIL derived T cells, we observed an increase in the uptake radioactivity in liver metastases of a patient with ovarian cancer. Neo^R marked TIL expanded in rI 2 can be detected in both man and in animals for a considerable time after injection (6,1C Previous studies in patients with melanoma have shown that TIL from these patients we successfully transduced with the neo^R gene using the LNL6 retroviral vector (5) which has the san backbone as G1Na, the vector proposed for our marker protocol. The neo^R transduced gene cel could be identified in biopsy material from melanoma metastases even at 64 days after injectio The **hypothesis** to be tested in this protocol is that ovarian rIL-2 expanded purified $CD8^+$ TI derived T cells concentrate in metastatic tumor sites in vivo after IV injection.

The experimental approach is briefly described as follows: Marking of the $CD8^+$ TIL-derived cells by infection with G1Na will be performed after positive selection has been accomplished, ar after the purified $CD8^+$ T cells are observed to be multiplying in a T-flask. At this point consentir patients will be registered. A mixture of bulk expanded transduced and nontransduced T cells w be injected IP with rIL-2. Following treatment with the adoptively transferred TIL, (containir transduced and nontransduced cells) and IP rIL-2, biopsies will be obtained at laparosco performed at 1 month after injection of the TIL, from previously documented tumor sites and fro normal tissues (peritoneum, omentum (if present) and lymph node tissue). Although animal ar human studies show that injected TIL can be recovered beyond 2 months, there is evidence th selection of an earlier time point will maximize neo^R detection, with the requirement of a small number of patients. DNA will be extracted by a proteinase K phenol procedure and the nec specific DNA quantitated by PCR according to Morgan et al (12). A successful result is defin when the mean number of marked cells in tumor tissue is significantly larger than the mea number of marked cells in blood or normal tissues. The study will be completed with a maximu of 9 and a minimum of 5 evaluable patients to determine the fold increase at metastatic tum sites. The proportions of $CD3^+CD8^+$ T cells will be determined in biopsies utilizing OKT8 and OK moAbs by histochemical procedures that we have established. In addition to these objectives, v will also determine the persistence of neo^R marked T cells in the abdominal cavity during the cour of treatment with TIL + rIL-2.